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TITLE OF THE INVENTION (280 characters max)

TUMOR NECROSIS FACTOR RECEPTOR FAMILY PROTEIN AND RELATED NUCLEIC ACID COMPOUNDS AND METHODS FOR THEIR USE

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Respectfully submitted,
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TUMOR NECROSIS FACTOR RECEPTOR FAMILY PROTEIN AND RELATED NUCLEIC ACID COMPOUNDS AND METHODS FOR THEIR USE

BACKGROUND OF THE INVENTION

This invention relates to a novel gene and its cognate protein, the protein being a member of the tumor necrosis factor receptor (TNFR) superfamily. Also contemplated are methods for identifying compounds that bind said receptor, methods for inhibiting osteoclast differentiation and bone resorption, methods for modulating the TNFR proteins and their respective ligand interactions where such interactions cause or exacerbate disease and methods for treating diseases.

The TNFR superfamily is a group of type I proteins (generally transmembrane) that share a conserved cysteine-rich motif, which is repeated three to six times in the extracellular domain (Smith, et al., 1994, Cell 76:953-62). Collectively, these repeat units form the ligand binding domains of these receptors (Chen et al., 1995, Chemistry 270:2874-78). The TNFR's are variably expressed in a variety of cell types, including but not limited to B cells, T cells, dendritic cells, and macrophages.

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The ligands for these receptors are a structurally related, yet distinct, group of proteins in the tumor necrosis factor (TNF) family. These ligands produce a variety of biological responses in TNFR-bearing cells, including proliferation, differentiation, immune regulation, inflammatory response, cytotoxicity, and apoptosis, binding to distinct but closely related receptors TNFR-1 and TNFR-2.

The nerve growth factor/TNFR superfamily continues to expand and includes, TNFR-1, TNFR-2, CD27, CD30, CD40, 4-1BB(CDw137, OX40, Fas (CD95), nerve growth factor receptor, Lymphotoxin-beta receptor(LTBR), Apo-3/DR3/Wsl-1lymphcyte-associated receptor of death(LARD)/TRAMP, DR4, DR5/TNF-related apoptosis inducing ligand (TRAIL)-R2, TRAIL recptor without an intracellular domain (TRID)/DcR1/TRAIL-R3 and osteoprotegrin (OPG). See Harrop J.A., 1998, J. Immunology, 161:1786-1794. Systemic delivery of TNF induces toxic shock and widespread tissue necrosis. Because of this, TNF may be responsible for the severe morbidity and mortality associated with a variety of infectious diseases, including sepsis.

Mutations in FasL, the ligand for the TNFR-related receptor FAS/APO (Suda et al., 1993, Cell 75:1169-78, are associated with autoimmunity (Fisher et al., 1995, Cell 81:935-46), while overproduction of FasL may be implicated in drug-induced hepatitis.

Soluble TNFR-1 receptors, and antibodies that bind TNF, have been tested for their ability to neutralize systemic TNFα (Leotsher et al., 1991, Cancer Cells 3(6):221-6). A naturally occurring form of a secreted TNFR-1 mRNA was

recently cloned, and its product tested for its ability to neutralize TNF activity *in vitro* and *in vivo* (Kohno et al., 1990, Proc. Nat. Acad, Sci. 87:8331-5).

TNF has also recently been implicated in the pathogenesis of bone loss induced by estrogen deficiency, presumably mediated by binding to certain members of the TNFR superfamily. Expression of a soluble TNFR-1/FcIgG3 fusion protein in transgenic ovariectomized mice was demonstrated to protect against the loss in bone mass and strength experienced by control animals (Ammann et al., 1997, J. Clin. Invest., 99:1699-1703). Moreover, a novel naturally-occurring secreted member of the TNFR superfamily were recently reported (by two separate groups) as having a role in regulating bone resorption (Simonet et al., 1997, Cell 89:309-19 (termed osteoprotegerin (OPG)); Tsuda et al., 1997, Biochem. and Biophys. Res. Comm. 234:137-42 (termed osteoclastogenesis inhibitory factor (OCIF))), functioning essentially as inhibitors of differentiation of bone-resorbing osteoclasts.

An object of the present invention is to identify new members of the TNFR superfamily. It is anticipated that new TNFR's may be transmembrane proteins or soluble forms thereof comprising extracellular domains. Indeed, the present invention relates to new nucleic acids and polypeptides encoded thereby that are closely related to TNFR-2, which are implicated in regulation of bone metabolism and numerous other conditions including, but not limited to, hepatitis, sepsis, renal failure, hepatocellular injury, cardiac ischemia, neuronal ischemia, transplantation

rejection, T cell overstimulation such as allergies, T cell depletion as in HIV, cancers such as colon carcinoma, melanoma, hepatocellular carcinoma, lung cancer, astrocytoma, esophageal carcinoma, and autoimmune diseases such as IDDM, multiple sclerosis (MS), rheumatoid arthritis and lupus.

It is another object of the present invention to identify novel interactions and/or binding between members of the TNFR superfamily and their respective ligands.

It is a further object of the present invention to identify the biological effects of interactions and/or binding between members of the TNFR superfamily and their respective ligands.

It is yet another object of the present invention to identify, prevent and/or treat conditions caused or exacerbated by undesired interactions and/or binding between members of the TNFR superfamily.and their respective ligands.

It is a still further object of the present invention to provide novel methods of modulating interactions and/or binding between members of the TNFR superfamily and their respective ligands.

SUMMARY OF THE INVENTION

The present invention provides isolated nucleic acid compounds and novel proteins functionally related to the tumor necrosis factor receptor (TNFR) superfamily. The nucleotide sequences and proteins described herein are referred to as "TNFRsol." TNFRsol protein does not include

any transmembrane domains and is, therefore, soluble. TNFRsol is also called OPG3 (osteoprotegrin 3) or TNFRsol/OPG3 and are used interchangeably herein. It is believed to be closely related to TNFR 6α and TNFR 6β discussed in WO98/30694 claiming priority to U.S.S.N. 60/035,496 the teachings of which are incorporated herein by reference.

Having the TNFRsol gene enables the production of recombinant TNFRsol/OPG3 protein, the isolation of orthologous genes from other organisms, and/or paralogous genes from the same organism, chromosome mapping studies, and the implementation of large scale screens to identify compounds and potential pharmaceutical agents that bind or regulate expression of said protein and modulate biological activity thereof.

In one embodiment, the present invention relates to an isolated nucleic acid compound encoding TNFRsol/OPG3 protein, or fragment thereof. A preferred nucleic acid compound comprises the nucleotide sequence identified as SEQ ID NO:1. Other preferred nucleic acid compounds comprise nucleotides 88-900 of SEQ ID NO:1 or nucleotides 102-536 of SEQ ID NO:1.

In another embodiment, the present invention relates to a nucleic acid that is at least 75% identical, and preferably at least 95% identical, to a nucleic acid that encodes SEQ ID NO:2, or fragment thereof.

In another embodiment, the present invention relates to a nucleic acid that hybridizes to the polynucleotide of SEQ ID NO:1, or fragments thereof, under

high stringency conditions and encodes a protein that is capable of inhibiting bone resorption.

In another embodiment, the present invention relates to a nucleic acid that hybridizes to the polynucleotide of SEQ ID NO:1, or fragments thereof, under low stringency conditions and encodes a protein that is capable of inhibiting bone resorption.

In another embodiment the present invention relates to an isolated protein molecule, or functional fragment thereof, wherein said protein molecule comprises the sequence identified as SEQ ID NO:2. Examples of functional fragments of preference include amino acid residues 30-300 of SEQ ID NO:2 or residues 34-195 of SEQ ID NO:2.

In yet another embodiment, the present invention relates to a recombinant DNA vector that incorporates SEQ ID NO:1, or fragments thereof, in operable linkage to gene expression sequences, enabling the gene to be transcribed and translated in a host cell.

In still a further embodiment, the present invention relates to a method for identifying compounds that bind a protein identified herein as SEQ ID NO:2, comprising the steps of admixing a substantially purified preparation of a protein comprising SEQ ID NO:2 with a test compound, and monitoring by any suitable means a binding interaction between said protein and said compound. This method may be employed with peptide fragments of SEQ ID NO:2

This invention also provides a method of determining whether a nucleic acid sequence of the present

invention, or fragment thereof, is present within a nucleic acid-containing sample, the method comprising contacting the sample under suitable hybridization conditions with a nucleic acid probe of the present invention.

This invention also provides a method for treating a patient in need of TNFRsol/OPG3 polypeptide activity comprising administering to said patient an effective amount of the polypeptide having the amino acid sequence of SEQ ID NO:2 including functional fragments comprising amino acid residues 30-300 of SEQ ID NO:2 or residues 34-195 of SEQ ID NO:2 wherein the patient is being treated for hepatitis, sepsis, renal failure, hepatocellular injury, cardiac ischemia, neuronal ischemia, transplantation rejection, allergies, HIV, colon carcinoma, melanoma, hepatocellular carcinoma, lung cancer, astrocytoma, esophageal carcinoma, IDDM, multiple sclerosis (MS), rheumatoid arthritis and lupus.

This invention further provides a method for modulating selected TNFR interactions with their respective TNFR ligands comprising administering and effective amount of the TNFRsol/OPG3 polypeptide wherein the TNFR family ligands are FasL and LIGHT.

This invention further provides a method for modulating selected TNFR interactions with their respective TNFR family ligands *in vitro* comprising administering an effective amount of the TNFRsol/OPG3 polypeptide.

This invention also provides a method for modulating selected TNFR interactions with their respective TNFR family ligands *ex vivo* comprising administering an effective amount

of the TNFRsol/OPG3 polypeptide, more particularly during gene therapy.

This invention further provides, among other things, a method for modulating FasL mediated apoptosis or LIGHT mediated cell proliferation *in vivo, in vitro and ex vivo*

Still further, the present invention provides a method for modulating TNFR interactions with their respective TNFR ligands which interactions occur in cells selected from the group consisting of T cells, myocytes, renal tubule epithelial cells (RTC), neutrophils, neurons, thyrocytes, stroma cells, acinar cells, Sertoli cells, macrophages, hepatocytes, leukemia cells, cells of the kidney cortex and tumor cells.

Still further the present invention provides a method of treating conditions such as hepatitis, sepsis, renal failure, hepatocellular injury, cardiac ischemia, neuronal ischemia, transplantation rejection, allergies, HIV, colon carcinoma, melanoma, hepatocellular carcinoma, lung cancer, astrocytoma, esophageal carcinoma, IDDM, multiple sclerosis (MS), rheumatoid arthritis and lupus by administering a pharmaceutical compositions.comprising the TNFRsol/OPG3 polypeptide.

DETAILED DESCRIPTION OF THE INVENTION

The terms "complementary" or "complementarity" as used herein refer to the capacity of purine and pyrimidine nucleotides to associate through hydrogen bonding to form

double stranded nucleic acid compounds. The following base pairs are related by complementarity: guanine and cytosine; adenine and thymine; and adenine and uracil. As used herein, "complementary" means that the aforementioned relationship applies to substantially all base pairs comprising two single-stranded nucleic acid compounds over the entire length of said molecules. "Partially complementary" refers to the aforementioned relationship in which one of two single-stranded nucleic acid compounds is shorter in length than the other such that a portion of one of the molecules remains single-stranded.

"Conservative substitution" or "conservative amino acid substitution" refers to a replacement of one or more amino acid residue(s) in a protein or peptide as stipulated in Table 1.

"Fragment thereof" refers to a fragment, piece, or sub-region of a nucleic acid or protein molecule whose sequence is disclosed herein, such that the fragment comprises 5 or more amino acids, or 10 or more nucleotides that are contiguous in the parent protein or nucleic acid compound. Fragment thereof may or may not retain biological activity. For example, a fragment of a protein disclosed herein could be used as an antigen to raise a specific antibody against the parent protein molecule. When referring to a nucleic acid compound, "fragment thereof" refers to 10 or more contiguous nucleotides, derived from the parent nucleic acid, and also, owing to the genetic code, to the complementary sequence. For example if the fragment entails the sequence 5'-AGCTAG-3', then "fragment

thereof" would also include the complementary sequence, 3'-TCGATC-5'.

The term "fusion protein" denotes a hybrid protein molecule not found in nature comprising a translational fusion or enzymatic fusion in which two or more different proteins or fragments thereof are covalently linked on a single polypeptide chain.

"Functional fragment" or "functionally equivalent fragment", as used herein, refers to a region, or fragment of a full length protein, or sequence of amino acids that, for example, comprises an active site, or any other conserved motif, relating to biological function. Functional fragments are capable of providing a biological activity substantially similar to a full length protein disclosed herein, namely the ability to inhibit differentiation of bone marrow stem cells into osteoclasts. Functional fragments may be produced by cloning technology, or as the natural products of alternative splicing mechanisms.

"Host cell" refers to any eukaryotic or prokaryotic cell that is suitable for propagating and/or expressing a cloned gene contained on a vector that is introduced into said host cell by, for example, transformation or transfection, or the like.

TNFRsol/OPG3 refers to a nucleic acid and a protein or amino acid sequence encoded thereby. TNFRsol/OPG3 is a member of the TNFR superfamily. This family of receptors mediates a variety of biological effects of TNF ligands, including but not cell proliferation, cell

differentiation, immune regulation, inflammatory response, cytotoxicity, apoptosis and limited to inhibition of bone resorption (by virtue of inhibiting osteoclast differentiation).

The term "homolog" or "homologous" describes the relationship between different nucleic acid compounds or amino acid sequences in which said sequences or molecules are related by partial identity or similarity at one or more blocks or regions within said molecules or sequences.

The term "hybridization" as used herein refers to a process in which a single-stranded nucleic acid compound joins with a complementary strand through nucleotide base pairing. The degree of hybridization depends upon, for example, the degree of homology, the stringency of hybridization, and the length of hybridizing strands. "Selective hybridization" refers to hybridization under conditions of high stringency.

"Isolated nucleic acid compound" refers to any RNA or DNA sequence, however constructed or synthesized, which is locationally distinct from its natural location.

A "nucleic acid probe" or "probe" as used herein is a labeled nucleic acid compound which hybridizes with another nucleic acid compound. "Nucleic acid probe" means a single stranded nucleic acid sequence that will combine with a complementary or partially complementary single stranded target nucleic acid sequence to form a double-stranded molecule. A nucleic acid probe may be an oligonucleotide or a nucleotide polymer. A probe will usually contain a

detectable moiety which may be attached to the end(s) of the probe or be internal to the sequence of the probe.

The term "orthologue" or "orthologous" refers to two or more genes or proteins from different organisms that exhibit sequence homology.

The term "paralogue" or "paralogous" refers to two or more genes or proteins within a single organism that exhibit sequence homology.

The term "plasmid" refers to an extrachromosomal genetic element. The plasmids disclosed herein are commercially available, publicly available on an unrestricted basis, or can be constructed from readily available plasmids in accordance with published procedures.

A "primer" is a nucleic acid fragment which functions as an initiating substrate for enzymatic or synthetic elongation of, for example, a nucleic acid compound.

The term "promoter" refers to a nucleic acid sequence that directs transcription, for example, of DNA to RNA. An inducible promoter is one that is regulatable by environmental signals, such as carbon source, heat, or metal ions, for example. A constitutive promoter generally operates at a constant level and is not regulatable.

"Recombinant DNA cloning vector" as used herein refers to any autonomously replicating agent, including, but not limited to, plasmids and phages, comprising a DNA molecule to which one or more additional DNA segments can or have been incorporated.

The term "recombinant DNA expression vector" or "expression vector" as used herein refers to any recombinant DNA cloning vector, for example a plasmid or phage, in which a promoter and other regulatory elements are present thereby enabling transcription of an inserted DNA, which may encode a protein.

The term "stringency" refers to hybridization conditions. High stringency conditions disfavor non-homologous basepairing. Low stringency conditions have the opposite effect. Stringency may be altered, for example, by temperature and salt concentration.

"Low stringency" conditions comprise, for example, a temperature of about 37° C or less, a formamide concentration of less than about 50%, and a moderate to low salt (SSC) concentration; or, alternatively, a temperature of about 50° C or less, and a moderate to high salt (SSPE) concentration, for example 1M NaCl.

"High stringency" conditions comprise, for example, a temperature of about 42° C or less, a formamide concentration of less than about 20%, and a low salt (SSC) concentration; or, alternatively, a temperature of about 65° C, or less, and a low salt (SSPE) concentration. For example, high stringency conditions comprise hybridization in 0.5 M $NaHPO_4$, 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65°C (Ausubel, F.M. *et al.* Current Protocols in Molecular Biology, Vol. I, 1989; Green Inc. New York, at 2.10.3).

"SSC" comprises a hybridization and wash solution. A stock 20X SSC solution contains 3M sodium chloride, 0.3M sodium citrate, pH 7.0.

"SSPE" comprises a hybridization and wash solution. A 1X SSPE solution contains 180 mM NaCl, 9 mM Na_2HPO_4, 0.9 mM NaH_2PO_4 and 1 mM EDTA, pH 7.4.

"Substantially pure" used in reference to a peptide or protein means that said peptide or protein is separated from other cellular and non-cellular molecules, including other protein molecules. A substantially pure preparation would be about at least 85% pure; preferably about at least 95% pure. A "substantially pure" protein as described herein could be prepared by a variety of techniques well known to the skilled artisan, including, for example, the IMAC protein purification method.

The term "vector" as used herein refers to a nucleic acid compound used for introducing exogenous or endogenous DNA into host cells. A vector comprises a nucleotide sequence which may encode one or more protein molecules. Plasmids, cosmids, viruses, and bacteriophages, in the natural state or which have undergone recombinant engineering, are examples of commonly used vectors.

The various restriction enzymes disclosed and described herein are commercially available and the manner of use of said enzymes including reaction conditions, cofactors, and other requirements for activity are well known to one of ordinary skill in the art. Reaction conditions for particular enzymes were carried out according to the manufacturer's recommendation.

The TNFRsol/OPG3 gene comprises a nucleotide sequence of 900 nucleotide base pairs (SEQ ID NO:1) that encodes a polypeptide of 300 amino acid residues in length (SEQ ID NO:2). The TNFRsol/OPG3 gene identified from colon cells has a 87 nucleotide base pair sequence at the 5' end (i.e., nucleotides 1-87 of SEQ ID NO:1) that encodes a 29 residue signal peptide (i.e., residues 1-29 of SEQ ID NO:2), which peptide is cleaved from the N-terminus upon secretion of the mature soluble protein (i.e., residues 30-300 of SEQ ID NO:2).

Those skilled in the art will recognize that owing to the degeneracy of the genetic code (i.e. 64 codons which encode 20 amino acids), numerous "silent" substitutions of nucleotide base pairs could be introduced into the sequence identified as SEQ ID NO:1 without altering the identity of the encoded amino acid(s) or protein product. All such substitutions are intended to be within the scope of the invention.

Also contemplated by the present invention are TNFRsol/OPG3 proteins and related functional fragments such as, for example, smaller alternatively spliced forms, or substitutions in which the primary sequence disclosed in SEQ ID NO:2 is altered by substitution or replacement or deletion or insertion at one or more amino acid positions, such that biological function is maintained. Functional fragments are conveniently identified as fragments of an intact TNFRsol/OPG3 protein that retain the capacity to inhibit osteoclast differentiation.

Several structural motifs have been identified within the primary sequence of TNFRsol/OPG3 protein that are thought to be important for biological function. For example, four cysteine rich motifs in the N-terminal domain, which are represented in a variety of related proteins, and which can form internal disulfide bonds, span from amino acid residue 34 to 195 of SEQ ID NO:2. It is presumed that this moiety retains biological function.

Functional analogs of the TNFRsol/OPG3 protein(s) are typically generated by deletion, insertion, or substitution of a single (or few) amino acid residues. Substitution modifications can generally be made in accordance with the following Table.

Table 1

ORIGINAL RESIDUE	EXEMPLARY SUBSTITUTIONS
ALA	SER
ARG	LYS
ASN	GLN, HIS
ASP	GLU
CYS	SER
GLN	ASN
GLU	ASP
GLY	PRO
HIS	ASN, GLN
ILE	LEU, VAL
LEU	ILE, VAL
LYS	ARG, GLN, GLU
MET	LEU, ILE
PHE	MET, LEU, TYR
SER	THR
THR	SER
TRP	TYR
TYR	TRP, PHE
VAL	ILE, LEU

Fragments of proteins

One embodiment of the instant invention provides fragments of the proteins disclosed that may or may not be

The present invention also provides fragments of the proteins disclosed herein wherein said fragments retain biological activity. As used herein, "functional fragments" includes fragments of SEQ ID NO:2 that retain and exhibit, under appropriate conditions, measurable biological activity, for example, the capacity to inhibit osteoclast differentiation.

Functional fragments of the proteins disclosed herein may be produced as described above, preferably using cloning techniques to engineer smaller versions of the intact gene, lacking sequence from the 5' end, the 3' end, from both ends, or from an internal site.

Gene Isolation Procedures

Those skilled in the art will recognize that the TNFRsol/OPG3 gene could be obtained by a plurality of recombinant DNA techniques including, for example, hybridization, polymerase chain reaction (PCR) amplification, or de novo DNA synthesis. (*See e.g.*, T. Maniatis *et al*. Molecular Cloning: A Laboratory Manual, 2d Ed. Chap. 14 (1989)).

Methods for constructing cDNA libraries in a suitable vector such as a plasmid or phage for propagation in prokaryotic or eukaryotic cells are well known to those skilled in the art. (*See e.g.* Maniatis *et al. Supra*). Suitable cloning vectors are well known and are widely available.

The TNFRsol/OPG3 gene or fragment thereof can be isolated from any tissue in which said gene is expressed.

In one method, mRNA is isolated from a suitable tissue, and first strand cDNA synthesis is carried out. A second round of DNA synthesis can be carried out for the production of the second strand. If desired, the double-stranded cDNA can be cloned into any suitable vector, for example, a plasmid, thereby forming a cDNA library. Oligonucleotide primers targeted to any suitable region of SEQ ID NO:1 can be used for PCR amplification of TNFRsol/OPG3. *See e.g.* PCR Protocols: A Guide to Method and Application, Ed. M. Innis et al., Academic Press (1990). The PCR amplification comprises template DNA, suitable enzymes, primers, and buffers, and is conveniently carried out in a DNA Thermal Cycler (Perkin Elmer Cetus, Norwalk, CT). A positive result is determined by detecting an appropriately-sized DNA fragment following agarose gel electrophoresis.

Protein Production Methods

One embodiment of the present invention relates to the substantially purified protein encoded by the TNFRsol/OPG3 gene.

Skilled artisans will recognize that the proteins of the present invention can be synthesized by a number of different methods, such as chemical methods well known in the art, including solid phase peptide synthesis or recombinant methods. Both methods are described in U.S. Patent 4,617,149, incorporated herein by reference.

The principles of solid phase chemical synthesis of polypeptides are well known in the art and may be found in general texts in the area. *See, e.g.*, H. Dugas and C.

Penney, Bioorganic Chemistry (1981) Springer-Verlag, New York, 54-92. For example, peptides may be synthesized by solid-phase methodology utilizing an Applied Biosystems 430A peptide synthesizer (Applied Biosystems, Foster City, CA) and synthesis cycles supplied by Applied Biosystems.

The proteins of the present invention can also be produced by recombinant DNA methods using the cloned TNFRsol/OPG3 gene. Recombinant methods are preferred if a high yield is desired. Expression of the cloned gene can be carried out in a variety of suitable host cells, well known to those skilled in the art. For this purpose, the TNFRsol/OPG3 gene is introduced into a host cell by any suitable means, well known to those skilled in the art. While chromosomal integration of the cloned gene is within the scope of the present invention, it is preferred that the gene be cloned into a suitable extra-chromosomally maintained expression vector so that the coding region of the TNFRsol/OPG3 gene is operably-linked to a constitutive or inducible promoter.

The basic steps in the recombinant production of the TNFRsol/OPG3 protein are:

a) constructing a natural, synthetic or semi-synthetic DNA encoding TNFRsol/OPG3 protein;

b) integrating said DNA into an expression vector in a manner suitable for expressing the TNFRsol/OPG3 protein, either alone or as a fusion protein;

c) transforming or otherwise introducing said vector into an appropriate eukaryotic or

prokaryotic host cell forming a recombinant host cell;

d) culturing said recombinant host cell in a manner to express the TNFRsol/OPG3 protein; and

e) recovering and substantially purifying the TNFRsol/OPG3 protein by any suitable means well known to those skilled in the art.

<u>Expressing Recombinant TNFRsol/OPG3 Protein in Prokaryotic and Eukaryotic Host Cells</u>

Prokaryotes may be employed in the production of recombinant TNFRsol/OPG3 protein. For example, the *Escherichia coli* K12 strain 294 (ATCC No. 31446) is particularly useful for the prokaryotic expression of foreign proteins. Other strains of *E. coli*, bacilli such as *Bacillus subtilis*, enterobacteriaceae such as *Salmonella typhimurium* or *Serratia marcescans*, various Pseudomonas species and other bacteria, such as *Streptomyces*, may also be employed as host cells in the cloning and expression of the recombinant proteins of this invention.

Promoter sequences suitable for driving the expression of genes in prokaryotes include β-lactamase [*e.g.* vector pGX2907, ATCC 39344, contains a replicon and β-lactamase gene], lactose systems [Chang et al., *Nature* (London), 275:615 (1978); Goeddel et al., *Nature* (London), 281:544 (1979)], alkaline phosphatase, and the tryptophan (trp) promoter system [vector pATH1 (ATCC 37695)], which is designed to facilitate expression of an open reading frame as a trpE fusion protein under the control of the trp

promoter. Hybrid promoters such as the tac promoter (isolatable from plasmid pDR540, ATCC-37282) are also suitable. Still other bacterial promoters, whose nucleotide sequences are generally known, may be ligated to DNA encoding the protein of the instant invention, using linkers or adapters to supply any required restriction sites. Promoters for use in bacterial systems also will contain a Shine-Dalgarno sequence operably-linked to the DNA encoding the desired polypeptides. These examples are illustrative rather than limiting.

The proteins of this invention may be synthesized either by direct expression or as a fusion protein comprising the protein of interest as a translational fusion with another protein or peptide which may be removed by enzymatic or chemical cleavage. It is often observed in the production of certain peptides in recombinant systems that expression as a fusion protein prolongs the lifespan, increases the yield of the desired peptide, or provides a convenient means of purifying the protein. This is particularly relevant when expressing mammalian proteins in prokaryotic hosts. A variety of peptidases (e.g. enterokinase and thrombin) which cleave a polypeptide at specific sites or digest the peptides from the amino or carboxy termini (*e.g.* diaminopeptidase) of the peptide chain are known. Furthermore, particular chemicals (*e.g.* cyanogen bromide) will cleave a polypeptide chain at specific sites. The skilled artisan will appreciate the modifications necessary to the amino acid sequence (and synthetic or semi-synthetic coding sequence if recombinant means are employed)

to incorporate site-specific internal cleavage sites. *See e.g.*, P. Carter, "Site Specific Proteolysis of Fusion Proteins", Chapter 13, in Protein Purification: From Molecular Mechanisms to Large Scale Processes, American Chemical Society, Washington, D.C. (1990).

In addition to prokaryotes, a variety of amphibian expression systems such as frog oocytes, and mammalian cell systems can be used. The choice of a particular host cell depends to some extent on the particular expression vector used. Exemplary mammalian host cells suitable for use in the present invention include HepG-2 (ATCC HB 8065), CV-1 (ATCC CCL 70), LC-MK_2 (ATCC CCL 7.1), 3T3 (ATCC CCL 92), CHO-K1 (ATCC CCL 61), HeLa (ATCC CCL 2), RPMI8226 (ATCC CCL 155), H4IIEC3 (ATCC CCL 1600), C127I (ATCC CCL 1616), HS-Sultan (ATCC CCL 1484), and BHK-21 (ATCC CCL 10), for example.

A wide variety of vectors are suitable for transforming mammalian host cells. For example, the pSV2-type vectors comprise segments of the simian virus 40 (SV40) genome required for transcription and polyadenylation. A large number of plasmid pSV2-type vectors have been constructed, such as pSV2-gpt, pSV2-neo, pSV2-dhfr, pSV2-hyg, and pSV2-β-globin, in which the SV40 promoter drives transcription of an inserted gene. These vectors are widely available from sources such as the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland, 20852, or the National Center for Agricultural Utilization Research, 1815 North University Street, Peoria, Illinois 61604-39999.

Promoters suitable for expression in mammalian cells include the SV40 late promoter, promoters from eukaryotic genes, such as, for example, the estrogen-inducible chicken ovalbumin gene, the interferon genes, the glucocorticoid-inducible tyrosine aminotransferase gene, the thymidine kinase gene promoter, and the promoters of the major early and late adenovirus genes.

Plasmid pRSVcat (ATCC 37152) comprises portions of a long terminal repeat of the Rous Sarcoma virus, a virus known to infect chickens and other host cells. This long terminal repeat contains a promoter which is suitable for use in the vectors of this invention. H. Gorman *et al.*, *Proc. Nat. Acad. Sci. (USA)*, 79, 6777 (1982). The plasmid pMSVi (NRRL B-15929) comprises the long terminal repeats of the Murine Sarcoma virus, a virus known to infect mouse and other host cells. The mouse metallothionein promoter has also been well characterized for use in eukaryotic host cells and is suitable for use in the present invention. This promoter is present in the plasmid pdBPV-MMTneo (ATCC 37224) which can serve as the starting material for the construction of other plasmids of the present invention.

Transfection of mammalian cells with vectors can be performed by a plurality of well known processes including, but not limited to, protoplast fusion, calcium phosphate co-precipitation, electroporation and the like. *See, e.g.*, Maniatis *et al.*, *supra*.

Some viruses also make appropriate vectors. Examples include the adenoviruses, the adeno-associated viruses, the vaccinia virus, the herpes viruses, the

baculoviruses, and the rous sarcoma virus, as described in U.S. Patent 4,775,624, incorporated herein by reference.

Eukaryotic microorganisms such as yeast and other fungi are also suitable host cells. The yeast *Saccharomyces cerevisiae* is the preferred eukaryotic microorganism. Other yeasts such as *Kluyveromyces lactis* and *Pichia pastoris* are also suitable. For expression in *Saccharomyces*, the plasmid YRp7 (ATCC-40053), for example, may be used. *See, e.g.*, L. Stinchcomb *et al.*, *Nature*, 282, 39 (1979); J. Kingsman *et al.*, *Gene*, 7, 141 (1979); S. Tschemper *et al.*, *Gene*, 10, 157 (1980). Plasmid YRp7 contains the TRP1 gene which provides a selectable marker for use in a trp1 auxotrophic mutant.

<u>Purification of Recombinantly-Produced TNFRsol/OPG3 Protein</u>

An expression vector carrying the cloned TNFRsol/OPG3 gene is transformed or transfected into a suitable host cell using standard methods. Cells that contain the vector are propagated under conditions suitable for expression of the recombinant TNFRsol/OPG3 protein. For example, if the recombinant gene has been placed under the control of an inducible promoter, suitable growth conditions would incorporate the appropriate inducer. The recombinantly-produced protein may be purified from cellular extracts of transformed cells by any suitable means.

In a preferred process for protein purification, the TNFRsol/OPG3 gene is modified at the 5' end to incorporate several histidine residues at the amino terminus of the TNFRsol/OPG3 protein. This "histidine tag" enables a single-step protein purification method referred to as

"immobilized metal ion affinity chromatography" (IMAC), essentially as described in U.S. Patent 4,569,794, which hereby is incorporated by reference. The IMAC method enables rapid isolation of substantially pure recombinant TNFRsol/OPG3 protein starting from a crude extract of cells that express a modified recombinant protein, as described above.

<u>Production of Antibodies</u>

The proteins of this invention and fragments thereof may be used in the production of antibodies. The term "antibody" as used herein describes antibodies, fragments of antibodies (such as, but not limited, to Fab, Fab', Fab_2', and Fv fragments), and chimeric, humanized, veneered, resurfaced, or CDR-grafted antibodies capable of binding antigens of a similar nature as the parent antibody molecule from which they are derived. The instant invention also encompasses single chain polypeptide binding molecules.

The production of antibodies, both monoclonal and polyclonal, in animals, especially mice, is well known in the art. *See, e.g.,* C. Milstein, <u>Handbook of Experimental Immunology</u>, (Blackwell Scientific Pub., 1986); J. Goding, <u>Monoclonal Antibodies: Principles and Practice</u>, (Academic Press, 1983). For the production of monoclonal antibodies the basic process begins with injecting a mouse, or other suitable animal, with an immunogen. The mouse is subsequently sacrificed and cells taken from its spleen are fused with myeloma cells, resulting in a hybridoma that reproduces *in vitro*. The population of hybridomas is

screened to isolate individual clones, each of which secretes a single antibody species, specific for the immunogen. Each antibody obtained in this way is the clonal product of a single B cell.

Chimeric antibodies are described in U.S. Patent No. 4,816,567, the entire contents of which is herein incorporated by reference. This reference discloses methods and vectors for the preparation of chimeric antibodies. An alternative approach is provided in U.S. Patent No. 4,816,397, the entire contents of which is herein incorporated by reference. This patent teaches co-expression of the heavy and light chains of an antibody in the same host cell.

The approach of U.S. Patent 4,816,397 has been further refined in European Patent Publication No. 0 239 400. The teachings of this European patent publication are a preferred format for genetic engineering of monoclonal antibodies. In this technology the complementarity determining regions (CDRs) of a human antibody are replaced with the CDRs of a murine monoclonal antibody, thereby converting the specificity of the human antibody to the specificity of a murine antibody.

Single chain antibodies and libraries thereof are yet another variety of genetically engineered antibody technology that is well known in the art. (*See, e.g.* R.E. Bird, *et al.*, *Science* 242:423-426 (1988); PCT Publication Nos. WO 88/01649, WO 90/14430, and WO 91/10737. Single chain antibody technology involves covalently joining the binding regions of heavy and light chains to generate a

single polypeptide chain. The binding specificity of the intact antibody molecule is thereby reproduced on a single polypeptide chain.

The antibodies contemplated by this invention are useful in diagnostics, therapeutics or in diagnostic/therapeutic combinations.

The proteins of this invention or suitable fragments thereof can be used to generate polyclonal or monoclonal antibodies, and various inter-species hybrids, or humanized antibodies, or antibody fragments, or single-chain antibodies. The techniques for producing antibodies are well known to skilled artisans. (*See e.g.* A.M. Campbell, Monoclonal Antibody Technology: Laboratory Techniques in Biochemistry and Molecular Biology, Elsevier Science Publishers, Amsterdam (1984); Kohler and Milstein, *Nature* 256, 495-497 (1975); Monoclonal Antibodies: Principles & Applications Ed. J.R.Birch & E.S. Lennox, Wiley-Liss, 1995.

A protein used as an immunogen may be modified or administered in an adjuvant, by subcutaneous or intraperitoneal injection into, for example, a mouse or a rabbit. For the production of monoclonal antibodies, spleen cells from immunized animals are removed, fused with myeloma cells, such as SP2/0-Ag14 cells, and allowed to become monoclonal antibody producing hybridoma cells in the manner known to the skilled artisan. Hybridomas that secrete a desired antibody molecule can be screened by a variety of well known methods, for example ELISA assay, western blot analysis, or radioimmunoassay (Lutz *et al. Exp. Cell Res.*

from a tissue that expresses the TNFRsol/OPG3 gene, suitable oligonucleotide primers complementary to SEQ ID NO:1 or to any sub-region therein, are prepared as described in U.S. Patent No. 4,889,818, hereby incorporated by reference. Other suitable protocols for the PCR are disclosed in PCR Protocols: A Guide to Method and Applications, Ed. Michael A. Innis et al., Academic Press, Inc. (1990). Using PCR, any region of the TNFRsol/OPG3 gene can be targeted for amplification such that full or partial length gene sequences may be produced.

The ribonucleic acids of the present invention may be prepared using polynucleotide synthetic methods discussed *supra,* or they may be prepared enzymatically, for example, using RNA polymerase to transcribe a TNFRsol/OPG3 DNA template.

The most preferred systems for preparing the ribonucleic acids of the present invention employ the RNA polymerase from the bacteriophage T7 or the bacteriophage SP6. These RNA polymerases are highly specific, requiring the insertion of bacteriophage-specific sequences at the 5' end of the template to be transcribed. *See*, Maniatis *et al.*, *supra*.

This invention also provides nucleic acids, RNA or DNA, that are complementary to SEQ ID NO:1, or fragments thereof.

Nucleic Acid Probes

The present invention also provides probes and primers useful for a variety of molecular biology techniques

including, for example, hybridization screens of genomic, subgenomic, or cDNA libraries, as well as hybridization against nucleic acids derived from cell lines or tissues that originate from drug-resistant tumors. Such hybridization screens are useful as methods to identify homologous and/or functionally related sequences from the same or other organisms, and further for investigating the mechanism by which drug resistance arises in various cancers. A nucleic acid compound comprising SEQ ID NO:1 or a complementary sequence thereof, or fragment thereof, which is at least 14 base pairs in length, and which will selectively hybridize to human DNA or mRNA encoding TNFRsol/OPG3 protein or fragment thereof, or a functionally related protein, is provided. Preferably, the 14 or more base pair compound is DNA. *See e.g.* B. Wallace and G. Miyada, "Oligonucleotide Probes for the Screening of Recombinant DNA Libraries," In *Meth. Enzym.*, 152, 432-442, Academic Press (1987).

Probes and primers can be prepared by enzymatic or recombinant methods, well known to those skilled in the art (*See e.g.* Sambrook et al. *supra*). A probe may be a single stranded nucleic acid sequence which is complementary in some particular degree to a nucleic acid sequence sought to be detected ("target sequence"). A probe may be labeled with a detectable moiety such as a radio-isotope, antigen, or chemiluminescent moiety. A description of the use of nucleic acid hybridization as a procedure for the detection of particular nucleic acid sequences is described in U.S. Patent No. 4,851,330 to Kohne, entitled "Method for

Detection, Identification and Quantitation of Non-Viral Organisms."

DNA sequence information provided by the present invention allows for the preparation of relatively short DNA (or RNA) sequences having the ability to specifically hybridize to gene sequences disclosed herein. In these aspects, nucleic acid probes of an appropriate length are prepared. The ability of such nucleic acid probes to specifically hybridize to a polynucleotide encoding a TNFRsol/OPG3 gene or related sequence lends particular utility in a variety of embodiments. Most importantly, the probes may be used in a variety of assays for detecting the presence of complementary sequences in a given sample.

In certain embodiments, it is advantageous to use oligonucleotide primers. The sequence of such primers is designed using a polynucleotide of the present invention for use in detecting, amplifying, or mutating a defined segment of a gene or polynucleotide that encodes a TNFRsol/OPG3 polypeptide using PCR technology.

Preferred nucleic acid sequences employed for hybridization studies, or assays, include probe molecules that are complementary to at least an about 14- to an about 70-nucleotide long stretch of a polynucleotide that encodes a TNFRsol/OPG3 polypeptide, such as the nucleotide base sequences designated as SEQ ID NO:1. A length of at least 14 nucleotides helps to ensure that the fragment is of sufficient length to form a duplex molecule that is both stable and selective. Molecules having complementary sequences over stretches greater than 14 bases in length are

percent GC content result in a Tm about 2°-10° C higher than the temperature at which the final assay will be performed. The base composition of the probe is also a significant factor because G-C base pairs exhibit greater thermal stability as compared to A-T base pairs. Thus, hybridization involving complementary nucleic acids of higher G-C content will be more stable at higher temperatures.

The ionic strength and incubation temperature under which a probe will be used should also be taken into account. It is known that hybridization will increase as the ionic strength of the reaction mixture increases, and that the thermal stability of molecular hybrids will increase with increasing ionic strength. On the other hand, chemical reagents such as formamide, urea, DMSO and alcohols, which disrupt hydrogen bonds, increase the stringency of hybridization. Destabilization of hydrogen bonds by such reagents can greatly reduce the Tm. In general, optimal hybridization for synthetic oligonucleotide probes of about 10-50 bases in length occurs approximately 5° C below the melting temperature for a given duplex. Incubation at temperatures below the optimum may allow mismatched base sequences to hybridize and can therefore result in reduced specificity.

The length of the target nucleic acid sequence and, accordingly, the length of the probe sequence can also be important. In some cases, there may be several sequences from a particular region, varying in location and length, which will yield probes with the desired hybridization

preferred nucleic acid vectors are those which comprise DNA, in particular SEQ ID NO:1, more particularly nucleotides 88-900 of SEQ ID NO:1, and more particularly nucleotides 102-585 of SEQ ID NO:1.

The skilled artisan understands that choosing the most appropriate cloning vector or expression vector depends upon a number of factors including the availability of restriction enzyme sites, the type of host cell into which the vector is to be transfected or transformed, the purpose of the transfection or transformation (e.g., stable transformation as an extrachromosomal element, or integration into the host chromosome), the presence or absence of readily assayable or selectable markers (e.g., antibiotic resistance and metabolic markers of one type and another), and the number of copies of the gene desired in the host cell.

Vectors suitable to carry the nucleic acids of the present invention comprise RNA viruses, DNA viruses, lytic bacteriophages, lysogenic bacteriophages, stable bacteriophages, plasmids, viroids, and the like. The most preferred vectors are plasmids.

When preparing an expression vector the skilled artisan understands that there are many variables to be considered, for example, whether to use a constitutive or inducible promoter. The practitioner also understands that the amount of nucleic acid or protein to be produced dictates, in part, the selection of the expression system. Regarding promoter sequences, inducible promoters are preferred because they enable high level, regulatable

expression of an operably-linked gene. The skilled artisan will recognize a number of suitable promoters that respond to a variety of inducers, for example, carbon source, metal ions, and heat. Other relevant considerations regarding an expression vector include whether to include sequences for directing the localization of a recombinant protein. For example, a sequence encoding a signal peptide preceding the coding region of a gene is useful for directing the extra-cellular export of a resulting polypeptide.

The present invention also provides a method for constructing a recombinant host cell capable of expressing proteins comprising SEQ ID NO:2, said method comprising transforming or otherwise introducing into a host cell a recombinant DNA vector that comprises an isolated DNA sequence that encodes SEQ ID NO:2. Of course. Such method also encompasses the host cells capable of expressing functional fragments of SEQ ID NO:2. The preferred host cell is any eukaryotic cell that can accommodate high level expression of an exogenously introduced gene or protein, and that will incorporate said protein into its membrane structure. Vectors for expression are those which comprise SEQ ID NO:1 or a fragment thereof. Transformed host cells may be cultured under conditions well known to skilled artisans such that SEQ ID NO:2 is expressed, thereby producing a recombinant TNFRsol/OPG3 protein in the recombinant host cell.

For the purpose of identifying compounds having utility as regulators or modifiers of bone resorption, it would be desirable to identify compounds that bind the

mg/kg/day. If given continuously, the TNFRsol/OPG3 polypeptide is typically administered at a dose rate of about 1 µg/kg/hour to about 50 µg/kg/hour, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution may also be employed. The length of treatment needed to observe changes and the interval following treatment for responses to occur appears to vary depending on the desired effect.

Pharmaceutical compositions containing the TNFRsol/OPG3 of the invention may be administered orally, rectally, parenterally, intracistemally, intravaginally, intraperitoneally, topically (as by powders, ointments, drops or transdermal patch), bucally, or as an oral or nasal spray. By "pharmaceutically acceptable carrier" is meant a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

The TNFRsol/OPG3 polypeptide is also suitably administered by sustained-release systems. Suitable examples of sustained-release compositions include semi-permeable polymer matrices in the form of shaped articles, e.g., films, or microcapsules. Sustained-release matrices include polylactides (U.S. Pat. No. 3,773.919, EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman, U. et al., *Biopolymers 22*:547-556 (1983)), poly (2-

hydroxyethyl methacrylate) (R.Langer et al., *J. Biomed. Mater. Res.* *15*:167-277 (1981), and R. Langer, *Chem. Tech.* *12*:98-105 (1982)), ethylene vinyl acetate (R. Langer et al., Id.) or poly-D- (-)-3-hydroxybutyric acid (EP 133,988). Sustained-release TNFRsol/OPG3 polypeptide compositions also include liposomally entrapped TNFRsol/OPG3 polypeptides. Liposomes containing TNFRsol/OPG3 polypeptides are prepared by methods known per se: DE 3,218,121; Epstein et al., Proc. Natl. Acad. Sci. (USA) 82:3688-3692 (1985); Hwang et al., Proc. Natl. Acad. Sci. (USA) 77:4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EDP 143,949; EP 142,641; Japanese Pat. Appl. 83-118008; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily, the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. percent cholesterol, the selected proportion being adjusted for the optimal TNFR polypeptide therapy.

For parenteral administration, in one embodiment, the TNFRsol/OPG3 polypeptide is formulated generally by mixing it at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a pharmaceutically acceptable carrier, i.e., one that is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation. For example, the formulation preferably does not include oxidizing agents and other compounds that are known to be deleterious to polypeptides.

Generally, the formulations are prepared by contacting the TNFRsol/OPG3 polypeptide uniformly and intimately with

liquid carriers or finely divided solid carriers or both. Then, if necessary, the product is shaped into the desired formulation. Preferably the carrier is a parenteral carrier, more preferably a solution that is isotonic with the blood of the recipient. Examples of such carrier vehicles include water, saline, Ringer's solution, and dextrose solution. Non-aqueous vehicles such as fixed oils and ethyl oleate are also useful herein, as well as liposomes.

The carrier suitably contains minor amounts of additives such as substances that enhance isotonicity and chemical stability, Such materials are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) polypeptides, e.g., polyarginine or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids, such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, manose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counterions such as sodium; and/or nonionic surfactants such as polysorbates, poloxamers, or PEG.

The TNFRsol/OPG3 polypeptide is typically formulated in such vehicles at a concentration of about 0.1 mg/ml to 100 mg/ml, preferably 1-10 mg/ml, at a pH of about 3 to 8. It

will be understood that the use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of TNFRsol/OPG3 polypeptide salts.

TNFRsol/OPG3 polypeptides to be used for therapeutic administration must be sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 micron membranes). Therapeutic TNFRsol/OPG3 polypeptide compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

TNFRsol/OPG3 polypeptides ordinarily will be stored in unit or multi-dose containers, for example, sealed ampoules or vials, as an aqueous solution or as a lyophilized formulation for reconstitution. As an example of a lyophilized formulation, 10-ml vials are filled with 5 ml of sterile-filtered 1% (w/v) aqueous TNFRsol/OPG3 polypeptide solution, and the resulting mixture is lyophilized. The infusion solution is prepared by reconstituting the lyophilized TNFRsol/OPG3 polypeptide using bacteriostatic Water-for-Injection.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human

administration. In addition, the polypeptides of the present invention may be employed in conjunction with other therapeutical compounds.

Methods of Using TNFRsol/OPG3

Another aspect of the present invention is the discovery that TNFRsol/OPG3 can specifically bind to Fas ligand and subsequently block Fas L-induced apoptosis. Fas ligand is a TNF family member protein and mainly expressed on activated T cells although as discussed further herein, it is expressed in other cells including myocytes, renal tubule epithelial cells (RTC), neutrophils, neurons, thyrocytes, stroma cells, acinar cells, Sertoli cells, macrophages, hepatocytes, leukemia cells, cells of the kidney cortex and tumor cells. Fas ligand (FasL) is also found in testis and eye and believed to be involved in immune privilege of these sites. While not intended to limit the present invention, it is believed that Fas ligand binds to its receptor, Fas, and trimerizes the receptor which subsequently binds to FADD and Caspase 8. Finally, caspases are activated and cells undergo to apoptosis.

Activated T cells express Fas ligand and induce apoptosis, a mechanism called activation induced cell death (AICD). As discussed in the Examples below, Jurkat cells, upon activation *in vitro* using anti-CD3 Ab, express FasL and undergo apoptosis. This activation induced cell death is inhibited by functional blocking anti-FasL Ab or caspase inhibitor (DEVE-fmk) as discussed in the Examples. Interestingly, the cell death mediated by FasL is also strongly inhibited by TNFRsol/OPG3, not OPG2. The specific

inhibition by TNFRsol/OPG3 is also shown by the soluble recombinant FasL induced Jurkat apoptosis not by OPG2 and OPG1(data not shown). Also, TNFRsol/OPG3 does not inhibit TRAIL- or TNFα-induced cell death further demonstrating that it does not inhibit all type of TNF-related apoptosis inducing ligands. It has been discovered that in dose dependent inhibition analysis the TNFRsol/OPG3 can inhibit anti-CD3 activation induced cell death at lowest concentration of 500 ng/ml. These evidence demonstrated that TNFRsol/OPG3 is a specific inhibitor for Fas L-mediated cells death through binding to Fas ligand.

FasL has been implicated in the damage caused to the liver in hepatitis. Use of the TNFRsol/OPG3 as a treatment for hepatitis is disclosed. In a mouse model of acute hepatitis, administration of anti-FasL antiboday to mice caused liver failure induced by apoptosis in hepatocytes and the animals die within hours. Kondo et al, 1997 Nature Medicine 3(4):409-413. See also Galle et al, J. Exp. Med, November 1995, 182:1223-1230

There are many additional diseases related to the FasL-Fas-mediated cell death. Insulin-dependent diabetes mellitus is a chronic autoimmune disease resulting from T-cell-mediated destruction of pancreatic β cells, which is mediated by FasL-Fas pathway. Fas reactivity has also been shown among the oligodendrocyte located along the lesion margin and in the adjacent white matter in acute and chronic multiple sclerosis (MS). FasL+ cells have been found in proximity to apoptotic Fas+ oligodendrocytes. In Hashimoto's thyroiditis (HT), it has been shown IL-1β

produced by activated macrophages induces massive Fas upregulation in thyrocyte. The consequent stimulation in thyrocyte of Fas and FasL induces thyrocyte apoptosis (DeMaria R et al, 1998, Immunol. Today 19:121). Another aspects of FasL-induced cell death pathway is the fact that many tumor cells including colon carcinoma, melanoma, hepatocellular carcinoma, lung cancer and astrocytoma and esophageal carcinoma, express biologically active Fas ligand and the FasL expression on tumor cells is associated with the increased apoptosis of tumor infiltrating lymphocytes, supporting the idea that FasL counterattack as a mechanism of immune privilege *in vivo* in human cancer (Bennett M et al. 1998, J. Immunol. 160:5669). Finally, FasL-mediated cell death is also shown to be involved in CD4+ T cell depletion (apoptosis) in HIV patient.

Thus, by inhibiting the FasL-Fas interaction TNFRsol/OPG3 can be used either alone or in combination with other therapeutics to prevent pancreatic β cell destruction in IDDM, to prevent thyrocyte death in HI disease, to prevent CD4+ T cell depletion in HIV patient and to treat tumors.

Still further, in addition to the discovery that TNFR receptor homologue TNFRsol/OPG3 interacts with TNF family ligand member, FasL, applicants have also discovered that TNFRsol/OPG3 intereacts with recently disclosed TNF family ligand, LIGHT (Mauri, D.M., (1998) Immunity, 8, 21-30). Since TNFRsol/OPG3 does not contain transmebrane and cytoplasmic domain, it is believed that TNFRsol/OPG3 works as an antagonist in the signalling pathway triggered by FasL

and LIGHT. This has an important implication since FasL and LIGHT signaling are involved in several diseases. For example, FasL binds cell surface receptor FAS and induce apoptosis of the FAS expressing cells. FAS expression was shown to be upregulated under several ischemic conditions, pointing to the fact that FAS may be an important mediator of apoptosis in various ischemic damages. (Kajstura J. et al. 1996, Laboratory Investigation, Vol. 74, No. 1, 86-107) Additionally, LIGHT has recently been identified as another TNF ligand family that is involved in T cell activation. LIGHT binds to a TNF receptor family called HVEM (Herpesvirus Entry Mediator) and activates proliferation signals. Data disclosed using the antagonist antibody against HVEM suggests that HVEM activation is an early event in the T cell activation cascade. (Harrop J.A. et al, 1998, J. Immunol.161:1786-1794. T cell activation of host is implicated in transplantation rejection of allografted tissues. A method of blocking LIGHT/HVEM signaling would be beneficial in preventing transplantation or other activated T cell mediated diseases.

Accordingly, also disclosed are methods of preventing neuronal/cardiac ischemic injury and preventing transplantation rejection by administering effective amounts of therapeutics comprising TNFRsol/OPG3.

The following examples more fully describe the present invention. Those skilled in the art will recognize that the particular reagents, equipment, and procedures described are merely illustrative and are not intended to limit the present invention in any manner.

EXAMPLE 1

RT-PCR Amplification of TNFRsol/OPG3 Gene from mRNA

A TNFRsol/OPG3 gene is isolated by reverse transcriptase PCR (RT-PCR) using conventional methods. Total RNA from a tissue that expresses the TNFRsol/OPG3 gene, for example, lung, is prepared using standard methods. First strand TNFRsol/OPG3 cDNA synthesis is achieved using a commercially available kit (SuperScript™ System; Life Technologies) by PCR in conjunction with specific primers directed at any suitable region of SEQ ID NO:1.

Amplification is carried out by adding to the first strand cDNA (dried under vacuum): 8 µl of 10X synthesis buffer (200 mM Tris-HCl, pH 8.4; 500 mM KCl, 25 mM $MgCl_2$, 1 ug/ul BSA); 68 µl distilled water; 1 µl each of a 10 uM solution of each primer; and 1 µl Taq DNA polymerase (2 to 5 U/µl). The reaction is heated at 94° C for 5 minutes to denature the RNA/cDNA hybrid. Then, 15 to 30 cycles of PCR amplification are performed using any suitable thermal cycle apparatus. The amplified sample may be analyzed by agarose gel electrophoresis to check for an appropriately-sized fragment.

EXAMPLE 2

Production of a Vector for Expressing TNFRsol/OPG3 in a Host Cell

An expression vector suitable for expressing TNFRsol/OPG3 or fragment thereof in a variety of prokaryotic host cells, such as *E. coli* is easily made. The vector

contains an origin of replication (Ori), an ampicillin resistance gene (Amp) useful for selecting cells which have incorporated the vector following a transformation procedure, and further comprises the T7 promoter and T7 terminator sequences in operable linkage to a TNFRsol/OPG3 coding region. Plasmid pET11A (obtained from Novogen, Madison WI) is a suitable parent plasmid. pET11A is linearized by restriction with endonucleases NdeI and BamHI. Linearized pET11A is ligated to a DNA fragment bearing NdeI and BamHI sticky ends and comprising the coding region of the TNFRsol/OPG3 gene as disclosed by SEQ ID NO:1 or a fragment thereof.

The TNFRsol/OPG3 gene used in this construction may be slightly modified at the 5' end (amino terminus of encoded protein) in order to simplify purification of the encoded protein product. For this purpose, an oligonucleotide encoding 8 histidine residues is inserted after the ATG start codon. Placement of the histidine residues at the amino terminus of the encoded protein serves to enable the IMAC one-step protein purification procedure.

EXAMPLE 3

Recombinant Expression and Purification of TNFRsol/OPG3 Protein

An expression vector that carries an open reading frame (ORF) encoding TNFRsol/OPG3 or fragment thereof and which ORF is operably-linked to an expression promoter is transformed into *E. coli* BL21 (DE3)(*hsd*S *gal* λcIts857 *ind*1*Sam*7*nin*5*lac*UV5-T7gene 1) using standard methods. Transformants, selected for resistance to ampicillin, are

chosen at random and tested for the presence of the vector by agarose gel electrophoresis using quick plasmid preparations. Colonies which contain the vector are grown in L broth and the protein product encoded by the vector-borne ORF is purified by immobilized metal ion affinity chromatography (IMAC), essentially as described in US Patent 4,569,794.

Briefly, the IMAC column is prepared as follows. A metal-free chelating resin (e.g. Sepharose 6B IDA, Pharmacia) is washed in distilled water to remove preservative substances and infused with a suitable metal ion [e.g. Ni(II), Co(II), or Cu(II)] by adding a 50mM metal chloride or metal sulfate aqueous solution until about 75% of the interstitial spaces of the resin are saturated with colored metal ion. The column is then ready to receive a crude cellular extract containing the recombinant protein product.

After removing unbound proteins and other materials by washing the column with any suitable buffer, pH 7.5, the bound protein is eluted in any suitable buffer at pH 4.3, or preferably with an imidizole-containing buffer at pH 7.5.

EXAMPLE 4

Tissue Distribution of TNFRsol/OPG3 mRNA

The presence of TNFRsol/OPG3 mRNA in a variety of human tissues was analyzed by Northern analysis. Total RNA from different tissues or cultured cells was isolated by a standard guanidine chloride/phenol extraction method, and

poly-A^{+} RNA was isolated using oligo(dT)-cellulose type 7 (Pharmacia). Electrophoresis of RNA samples was carried out in formaldehyde followed by capillary transfer to Zeta-Probe™ nylon membranes (Bio-Rad, Hercules, Calif.). SEQ ID NO:1 was the template for generating probes using a MultiPrime™ random priming kit (Amersham, Arlington Heights, Ill.). The efficiency of the labeling reaction was approximately 4×10^{10} cpm incorporated per µg of template. The hybridization buffer contained 0.5M sodium phosphate, 7% SDS (wt/vol), 1% BSA (wt/vol), and 1 mM EDTA. Prehybridization was carried out in hybridization buffer at 65° C for 2 h and ^{32}P-labeled probe was added and incubation continued overnight. The filters were washed in Buffer A (40 mM sodium phosphate pH 7.2, 5% SDS [wt/vol], 0.5% BSA [wt/vol], and 1 mM EDTA) at 65° C for 1 h, and then in Buffer B (40 mM sodium phosphate, pH 7.2, 1% SDS [wt/vol], and 1 mM EDTA) at 65° C for 20 minutes. The filters were air-dried and exposed to Kodak X-OMAT AR film at -80° C with an intensifying screen.

The results showed that TNFRsol/OPG3 mRNA was present in numerous tissues, including stomach, spinal cord, lymph node, trachea, spleen, and lung.

EXAMPLE 5

Production of an Antibody to a Protein

Substantially pure protein or fragment thereof is isolated from transfected or transformed cells using any of the well known methods in the art, or by a method specifically disclosed herein. Concentration of protein in

a final preparation is adjusted, for example, by filtration through an Amicon filter device such that the level is about 1 to 5 ug/ml. Monoclonal or polyclonal antibody can be prepared as follows.

Monoclonal antibody can be prepared from murine hybridomas according to the method of Kohler and Milstein (*Nature*, 256, 495, 1975), or a modified method thereof. Briefly, a mouse is repetitively inoculated with a few micrograms of the protein or fragment thereof, or fusion peptide thereof, over a period of a few weeks. The mouse is then sacrificed and the antibody producing cells of the spleen isolated. The spleen cells are fused by means of polyethylene glycol with mouse myeloma cells. Fused cells that produce antibody are identified by any suitable immunoassay, for example, ELISA, as described in E. Engvall, *Meth. Enzymol.*, 70, 419, 1980.

Polyclonal antiserum can be prepared by well known methods (*See e.g.* J. Vaitukaitis *et.al. Clin. Endocirnol. Metab*. 33, 988, 1971) that involve immunizing suitable animals with the proteins, fragments thereof, or fusion proteins thereof, disclosed herein. Small doses (e.g. nanogram amounts) of antigen administered at multiple intradermal sites appears to be the most reliable method.

EXAMPLE 6

Murine Osteoclast Differentiation Assay

The co-culture method of Takahashi et al. (Endocrinology 123:2600 1988) was modified as described in Galvin et al. (Endocrinology 137:2457 1996) and used to

study the effects of various agents on osteoclast differentiation.

Male Balb/C mice (4-8 weeks old) were euthanized with CO_2, the femurs removed, and the marrow flushed out of the femurs with growth medium. Bone marrow cells were pelleted by centrifugation at 500 x g for 6 min. and resuspended in the growth medium (RPMI 1640 plus 5% heat inactivated fetal bovine-serum and 1% antibiotic-antimycotic solution). The marrow population (5×10^4 cells/cm^2) was seeded in tissue culture dishes in which BALC cells (a stable cell line derived from neonatal mouse calvariae, 1.5×10^4 cells/cm^2) had been plated 2 h prior to addition of bone marrow. The cells were cultured for 7 days in a humidified incubator at 37°C with 5% CO_2 with medium changes on days 3 and 5. Cultures were treated with or without 10^{-8}M 1,25-$(OH)_2D_3$ on days 0, 3, and 5. In addition, the cells were treated with or without secreted TNFRsol/OPG3 protein purified from the conditioned medium of cells transfected with TNFRsol/OPG3 gene (SEQ ID NO:1). Following 7 days of culture, the cells in 24-well cluster dishes were fixed with formalin (3.7% for 10 min) and then stained for tartrate-resistant acid phosphatase (TRAP) using a modification of the method described by Graves, L and Jilka RL, J Cell Physiology 145:102 1990. The number of osteoclasts (TRAP-positive cells containing 3 or more nuclei) was quantitated. Results are reported in Table 1.

Table 1

TNFRsol/OPG3 (ng/ml)	Osteoclasts/well[a]
0.00	145.50 ± 7.33
0.01	40.50 ± 2.39*
0.10	65.50 ± 3.33*
1.00	97.50 ± 3.10*
10.00	170.17 ± 8.26
100.00	335.00 ± 8.90*

a - Each value represents the mean and standard error of 6 wells.
*$p<0.05$ compared to control group

EXAMPLE 7

Porcine Osteoclast Differentiation Assay

Neonatal pigs (aged 1-5 days) were euthanized with CO_2, the appendages were rinsed with 70% ethanol, the soft tissues were removed, and the humeri, radii, ulnae, femora, tibiae and fibulae were excised. The long bones were placed in ice-cold calcium and magnesium-free Hank's balanced salt solution (CMF-HBSS, Gibco BRL) and cleaned of all soft tissues. The bones were split longitudinally and the endosteal surfaces were scraped to remove both the marrow and trabecular bone. The suspension of trabecular bone particles and marrow cells was agitated by vigorous shaking

and passed through a 200 mm and then 100 mm sieve. Cells were centrifuged at 500 x g for 10 minutes at 4°C, the pellet was resuspended in CMF-HBSS, and then separated on a Ficoll-Paque gradient (Pharmacia, Piscataway, NJ). The mononuclear cell fraction from the gradient was washed twice in CMF-HBSS and passed through a 35 mm sieve. The cells were suspended in growth medium consisting of a-MEM (pH 7.2, which was modified to contain 8.3 mM $NaHCO_3$ (Gibco BRL, Grand Island, NY)), 10% heat-inactivated fetal bovine serum (FBS, Hyclone, Logan, UT) and 2% antibiotic/antimycotic solution (Gibco BRL, Grand Island, NY) and seeded onto tissue culture dishes at a density of 1×10^6 cells/cm^2. A typical marrow cell yield was between $1\text{-}2 \times 10^9$ cells/animal, which varied with the size of the animal. The cells were incubated at 37°C in a humid incubator with 5% CO_2. After 24-48 h, nonadherent cells were removed and seeded in either 24-well cluster dishes at a density of 7.5 $\times 10^5$ cells/cm^2 in growth medium which did or did not contain 10^{-8} M 1,25-$(OH)_2D_3$ (Biomol, Plymouth Meeting, PA) and TNFRsol/OPG3 protein (obtained as in Example 6). Cells were cultured for up to 10 days with medium changes every 48-72 h with growth medium that did or did not contain 1,25-$(OH)_2D_3$ and TNFRsol/OPG3 protein. Following 5 days of culture, the cells were fixed with formalin (3.7% for 10 min) and then stained for tartrate-resistant acid phosphatase (TRAP) as in Example 6. The number of osteoclasts (TRAP-positive cells containing 3 or more nuclei) was quantitated. Results are reported in Table 2.

Table 2

TNFRsol/OPG3 (ng/ml)	Osteoclasts/well[a]
0.00	214.83 + 14.22
0.01	68.83 + 6.28*
0.10	176.17 + 23.01
1.00	228.50 + 17.26
10.00	228.50 + 29.29
100.00	382.33 + 26.59*

a Each value represents the mean and standard error of 6 wells.
*p<0.05 compared to control group

EXAMPLE 8

<u>Construction of TNFRsol/OPG3-Flag Expression Vector</u>

To facilitate confirmation of TNFRsol/OPG3 expression (without the use of antibodies), a bicistronic expression vector (pIG1-TNFRsol/OPG3F) was constructed by insertion of an "internal ribosome entry site"/enhanced green fluorescent protein (IRES/eGFP) PCR fragment into the mammalian expression vector pGTD (Gerlitz, B. et al., 1993, Biochemical Journal 295:131). This new vector, designated pIG1, contains the following sequence landmarks: the E1a-responsive GBMT promoter (D. T. Berg et al., 1993 BioTechniques 14:972; D.T. Berg et al., 1992 Nucleic Acids Research 20:5485); a unique *BclI* cDNA cloning site; the IRES

sequence from encephalomyocarditis virus (EMCV); the eGFP (Clontech) coding sequence (Cormack, et al., 1996 Gene 173:33); the SV40 small "t" antigen splice site/poly-adenylation sequences; the SV40 early promoter and origin of replication; the murine dihydrofolate reductase (dhfr) coding sequence; and the pBR322 ampicillin resistance marker/origin of replication.

Based upon the human TNFRsol/OPG3 sequence, the following primers were synthesized: 5'- TAGGGCTGATCAAGGATGG GCTTCTGGACTTGGGCGGCCCCTCCGCAGGCGGACCGGGG-3' (SEQ ID NO:3); and 5'- AGGGGGGCGGCCGCTGATCATCACTTGTCGTCGTCGTCCTTGTAGTCGTGCA CAGGGAGGAAGCGC - 3' (SEQ ID NO:4). The reverse primer contained the Flag epitope sequence (nucleotides 24-47 of SEQ ID NO:4) (Micele, R.M. et al., 1994 J. Immunol. Methods 167:279). These primers were then used to PCR amplify the TNFRsol/OPG3 cDNA. The resultant 1.3 Kb PCR product was then digested with *BclI* (restriction sites incorporated into primers, underlined above) and ligated into the unique *BclI* site of pIG1 to generate the plasmid pIG1-TNFRsol/OPG3F. The human TNFRsol/OPG3 cDNA orientation and nucleotide sequence were confirmed by restriction digest and double stranded sequencing of the insert.

EXAMPLE 9

Construction of TNFRsol/OPG3-non-Flag Expression Vector

In order to generate a non-Flagged expression vector (pIG1-TNFRsol/OPG3), the 24-base DNA sequence encoding the eight amino acid FLAG epitope was deleted from the pIG1-TNFRsol/OPG3F construct using the Quik Change

population were sorted into a pool and as single cells. The high fluorescence pools were subjected to three successive sorting cycles. Pools and individual clones from the second and third cycles were analyzed for TNFRsol/OPG3 production by SDS-PAGE. Pools or clones expressing TNFRsol/OPG3 at the highest level judged from Coomassie staining were used for scale-up and TNFRsol/OPG3 purification.

EXAMPLE 11

Large Scale TNFRsol/OPG3 Protein Purification

Large scale production of TNFRsol/OPG3 was done by first growing the stable clones in several 10 liter spinners. After reaching confluency, cells were further incubated for 2-3 more days to secrete maximum amount of TNFRsol/OPG3 into media. Media containing TNFRsol/OPG3 was adjusted to 0.1% CHAPS and concentrated in an Amicon ProFlux M12 tangential filtration system to 350 ml. The concentrated media was centrifuged at 19,000 rpm (43,000 x g) for 15 minutes and passed over a SP-5PW TSK-GEL column (21.5 mm x 15 cm; TosoHaas) at a flow rate of 8 ml/min. The column was washed with buffer A(20 mM MOPS, 0.1% CHAPS, pH 6.5) until the absorbency (280 nm) returned to baseline and the bound proteins were eluted with a linear gradient from 0.1 M-0.3 M NaCl(in buffer A) developed over 85 min. Fractions containing TNFRsol/OPG3 were pooled and passed over a (7.5 mm x 7.5 cm) Heparin-5PW TSK-GEL column equilibrated in buffer B (50 mM Tris, 0.1% CHAPS, 0.3 M NaCl, pH 7.0). The bound protein was eluted with a linear gradient from 0.3 M-1.0 M NaCl (in buffer B) developed over 60 min. Fractions

containing TNFRsol/OPG3 were pooled and passed over a 1 cm x 15 cm Vydac C4 column equilibrated with 0.1% TFA/H_20. The bound TNFRsol/OPG3 was eluted with a linear gradient from 0-100% CH_3CN/0.1% TFA. Fractions containing TNFRsol/OPG3 were analyzed by SDS-PAGE and found to be greater than 95% pure and were dialyzed against 8 mM $NaP0_4$, 0.5 M NaCl, 10% glycerol, pH 7.4. The N-terminal sequence of TNFRsol/OPG3 was confirmed on the purified protein. Mass spectral analysis and Endogylcosidase-F digestion indicates that TNFRsol/OPG3 is glycosylated.

EXAMPLE 12

FAS LIGAND BINDING EXPERIMENTS

To detect TNFRsol/OPG3 interaction with FasL.

Dot blot experiment was performed to scan known TNF ligands that are commercially available TRAIL and FasL for interaction with TNFRsol/OPG3.

TRAIL (RnD Systems) and FasL (Kamiya Biomedical Company) were spotted on a nitrocellulose paper and incubated with purified TNFRsol/OPG3-Flag. TNFRsol/OPG3 was washed away and binding TNFRsol/OPG3 was detected using anti Flag antibody

Both OPG2Fc and TNFRsol/OPG3Flag were overexpressed and purified according the examples above. The filter paper was subsequently blocked for 30 min using 5% nonfat milk in PBS in room temperature.

The nitrocellulose paper was subsequently mixed with the cell lysate containing FasL-Myc, and further incubated on a rotator for 1 hour at room temperature. Secondary and

tertiary incubations were performed with anti-myc antibody and anti-mouse IgG-HRP for 1 hour and 30 minutes respectively. The protein containing myc epitope was detected by chemiluminescence on X-ray film which showed that OPG3 bound to FasL specifically.

First a baseline experiment was done for the Fas-FasLigand interaction *in vitro*. Unless otherwise indicated, all washing steps use TBST (Tris Buffer Saline with Tween 20 from SIGMA) and were done 3 to 6 times.

mrecFas (100 ng) was adsorbed on to ELISA plate. Then the plate was is blocked by TBST plus 0.1%Gelatine. Thereafter, hFasLigand (Flag-tagged) was added at different concentrations with a maximum concentration of 300 ng going down to 1 ng on TBST plus a 0.1% solution containing 1 micrograms/ml of M2 Abs (antiflag antibodies purchased through Scientific Imaging System division of Kodak). After washing the plate 6 times, anti-mouse-Abs-HRP (3000 dilution, Bio-Rad) was added to the wells. After washings three times, visualization enzymatic reaction using ABTS as a substrate was performed. Unless otherwise noted, an ELISA reader (Molecular Devices, Corp., Menlo Park, California) was used.

The following data was collected:

FasL, ng	OD, 405nM
1	.1
5	.2
10	.3
50	.7
100	1.2

500	1.6

OPG3 prevents Fas-FasLigand interaction

As above, mrecFas (100 ng) was adsorbed on to ELISA plate. Again the plate was blocked by TBST and 0.1%Gelatine. Thereafter, hFasLigand (Flag-tagged, 30 ng per each point) in the presence of different OPG3 concentrations (Max concentration 300 ng down to 1 ng) on TBST plus a 0.1% solution containing 1 microgram/ml of M2 Abs is added to each well. As before, after washing of the plate, anti-mouse-Abs-HRP (3000 dilution, Bio-Rad) was added to the wells. After washings, visualization ezymatic reaction using ABTS as a substrate.was performed. The data is shown in the following table.

TNFRsol/OPG3, ng	OD, 405nM
1	0.36
5	0.36
10	0.36
50	0.28
100	0.18
500	0.06

FasLigand binds Fas and OPG3 with different affinities.

OPG3 and Fas (100 ng of each) were adsorbed on to an ELISA plate. hFasLigand (Flag-tagged) was added at different concentrations to a maximum concentration of 300 ng down to 0.1 ng on TBST plus a 0.1% solution containing 1 microgram/ml of M2 Abs. After washing of the plate, anti-mouse-Abs-HRP (1:3000 dilution, Bio-Rad)was added to the wells. After washings, visualization enzymatic reaction

using ABTS as a substrate was performed. The table below shows the data.

FasL, ng	TNFRsol/OPG3 OD	Fas OD, 405nM
0.1	0	0
0.5	0	0
1.0	.02	0
5.0	.04	.01
10	.12	.03
50	.28	.045
100	.78	.18

EXAMPLE 13

Measuring the effect of TNFRsol/OPG3 on anti-CD3 induced Jurkat apoptosis

Non-tissue treated 24 well plates (Decton Dickinson, Mansfield, MA) were coated with 0.5 ml of 1 ug/ml anti-CD3 (Farmingen) in PBS for 90 min at 37 °C. The plate was washed once with PBS. 1 ml of 1 X 10^6 cell/ml was seated in each well with or without following treatment: 10 µM DEVD-cmk, 1 ug OPG2-Fc, 1 or 2 ug of OPG3 and 1 ug anti FasL Ab.

Cells were incubated overnight at 37 °C incubator and cells were then stained by Annexin V and PI staining. Apotosis was analysed by flow cytometer (FACS). Cell apoptosis was indicated by positive staining with Annexin V.

Control Jurkat	6.97
Jurkat + anti Fas	59.28
Jurkat + antiCD3	46.32
Jurkat + antiCD3 + DEVDcmk	30.80
Jurkat + antiCD3 + OPG3 (1ug)	27.77

Jurkat + antiCD3 + OPG2-Fc (1ug)	45.78
Jurkat + antiCD3 + OPG3 (2ug)	18.67
Jurkat + antiCD3 + antiFasL Ab	24.05

EXAMPLE 14

Measuring the effect of TNFRsol/OPG3 on recombinant FasL induced Jurkat cells apoptosis

1 ml of 1 X 10^6 cell/ml was added into each well of 24 well tissure culture plate and treated with following reagents: soluble Fas L (200 ng), Fas L plus 1 ug TNFRsol/OPG3, Fas L plus 1 ug OPG2-Fc, Trail (200 ng), Trail plus 1 ug TNFRsol/OPG3. Cells were incubated overnight at 37 °C and then stained with Annexin V and PI. Cell apoptosis was analysed by flow cytometer (FACS).

Control Jurkat	3.23
Jurkat + FasL (200ng/ml)	67.39
Jurkat + FasL (200ng/ml) + anti FasL Ab (1 ug)	3.3
Jurkat + FasL (200ng/ml) + TNFRsol/OPG3 (1 ug)	3.32
Jurkat + FasL (200ng/ml) + TNFRsol/OPG3 (1 ug)	4.6
Jurkat + FasL (200ng/ml) + OPG2(1ug)	70.58
Jurkat + FasL (200ng/ml) + OPG2(1ug)	69.58
Jurkat + TRAIL (200ng/ml)	17.47
Jurkat + TRAIL (200ng/ml)	17.43

EXAMPLE 15

Measuring the effect of TNFRsol/OPG3 in a dose-dependent manner on anti-CD3 induced Jurkat apoptosis

The same steps for plate coating and cell treatment set out in Example 13 were followed except a different amount of TNFRsol/OPG3 was added into each well. The following table indicates the amounts added:

Jurkat cells (Control)	5.33
Jurkat cells + anti CD3	27.49
Jurkat cells + anti CD3 + anti FasL neutralization Ab	12.74
Jurkat cells + anti CD3 + OPG2-Fc 4ug	26.24
Jurkat cells + anti CD3 + TNFRsol/PG3 3000ng	14.68
Jurkat cells + anti CD3 + TNFRsol/OPG3 2000ng	17.02
Jurkat cells + anti CD3 + TNFRsol/OPG3 1000ng	24.29
Jurkat cells + anti CD3 + TNFRsol/OPG3 500ng	27.48
Jurkat cells + anti CD3 + TNFRsol/OPG3 250ng	28.93
Jurkat cells + anti CD3 + TNFRsol/OPG3 125ng	29.4
Jurkat cells + anti CD3 + TNFRsol/OPG3 62.5ng	28.99
Jurkat cells + anti CD3 + TNFRsol/OPG3 31.25ng	28.21
Jurkat cells + anti CD3 + TNFRsol/OPG3 15.625ng	28.80

EXAMPLE 17

Measuring the effect of human TNFRsol/OPG3 on murine Fasl-mediated apoptosis using mouse T cell hybridoma cells (LTT cells (Cytotoxicity Assay)

The same steps as in Example 16 were followed on Day 1 and Day 2. On Day 3 20ul of MTS solution (Promega) was added to the cells which were then incubated at 37°C for 2 hours. Using a plate reader, the absorbances at 490nm wavelength were collected.

Anti-CD3 conc. (ug/ml)	s-Fas (1ug/ml)	OPG3 (1ug/ml)	Anti-FasL	Control
0	1.774	1.691	2.01	1.534
0.0014	1.968	1.923	2.134	1.614
0.004	1.929	1.982	2.147	1.653
0.012	1.779	2.006	2.108	1.284
0.037	1.777	2.006	1.988	0.834
0.11	1.638	1.874	1.956	0.733
0.33	1.624	1.671	1.978	0.648
1	1.459	1.581	1.887	0.664

EXAMPLE 18

LIGHT Binding experiments

To confirm the dot blot binding between LIGHT and TNFRsol/OPG3, 293 cells were transiently transfected with LIGHT expression construct overnight. Next day cells were detached and incubated with TNFRsol/OPG3-Flag on ice. The Flag epitope was subsequently detected by anti-Flag conjugated with fluorochrome and the cells population that shows specific binding with TNFRsol/OPG3 was detected by flow cytometer. As a control we used vector transfected cells. To make sure that the binding was specific, a competition assay with 10 fold access of untagged TNFRsol/OPG3 was performed.

More particularly, 6 well dishes of cells transfected as above. Both vector and m-LIGHT expressing cells were provided. Cells were detached from the plates by vigorous pipetting with a P100 Pipettor. Then, in PBS/BSA, 0.1%, cells were exposed to either:

a. GST/flag, TNFRsol/OPG3/flag, or HVEM at 20 nM or;

b. TNFRsol/OPG3/flag at 20 nM + either GST/flag, TNFRsol/OPG3, HVEM at 200 nM or;

c. TNFRsol/OPG3/flag at 20 nM + HVEM + anti-human FAS ligand at 200 nM;

d. anti-human FAS ligand-biotin at 1 μg/ml;

Cells were incubated on ice for 30 minutes and washed with PBS/BSA, 0.1%. Cells were then exposed to either anti-human IgG-biotin, 1μg/ml (for detection of HVEM), M2-biotin, 2 μg/ml (for detection of flag conjugates). Cells were incubated on ice for 30 minutes and washed with PBS/BSA,

0.1%. Thereafter, cells were exposed to streptavidin Alexa 488 (SIGMA) at a 1:1000 dilution. Again, cells were incubated on ice for 30 minutes and washed with PBS/BSA, 0.1%. Cells were analyzed using a FACSORT flow cytometer (Decton Dickinson) to determine binding.

The cell surface binding assay using flow cytometer confirmed that peaks shifted only when LIGHT expressing cells were stained with TNFRsol/OPG3-Flag was used (data not shown). There was no shift in the control cells when stained with TNFRsol/OPG3-Flag. The shifted peak was completely reversed to baseline peak when the cells were preincubated with 10 fold excess of non tagged TNFRsol/OPG3 thereby preoccupying all the binding sites for TNFRsol/OPG3-Flag.

EXAMPLE 19

■ In vivo testing of TNFRsol/OPG3 for treatment of IDDM

To test the functions of TNFRsol/OPG3 diabetes *in vivo*, an IDDM mouse model for example NOD mice is used. Using NOD mice, the mellitus of mouse pancreatic β cells is induced. One half the mice are treated with TNFRsol/OPG3 while the other half are not. The progression of disease is studied by examination of pancreatic β cells which have be treated in the presence and absence of TNFRsol/OPG3 once the disease is induced. In the animals treated with TNFRsol/OPG3, a reduction in the destruction of mouse pancreatic β cells is expected.

EXAMPLE 20

In vivo testing of TNFRsol/OPG3 for treatment of Tumors

To test the effect of TNFRsol/OPG3 on tumor cells *in vivo,* tumor cells are injected into mice. One half the mice are treated with TNFRsol/OPG3 and the other half are not. Tumor progress is measured both in the presence and absence of TNFRsol/OPG3. Administration of TNFRsol/OPG3 is expected to prevent increase tumor cell death and/or arrest tumor progression.

EXAMPLE 21

In vivo testing of TNFRsol/OPG3 for treatment of Chronic Renal Failure

Using the p53 mouse as set out in Schelling, J.R. et al, (1998) Laboratory Investigation 78(7):813-824, renal failure is induced. One half the mice are treated with TNFRsol/OPG3 and the other half are not. The progress of the renal failure is measured both in the presence and absence of TNFRsol/OPG3. Administration of TNFRsol/OPG3 is expected to prevent acute lethality and renal failure.

EXAMPLE 22

In vivo testing of TNFRsol/OPG3 for treatment of Liver Damage

Using a mouse or rat, a model of liver damage is induced using the methods set out in Tsuji H., et al, 1997, Infection and Immunity, 65(5):1892-1898. After priming with heat killed bacteria such as *Propionibacterium acnes,* challenge with a low dose of lipopolysaccaride (LPS) induces acute and massive hepatic injury. Administration of

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Dou, Shenshen
Song, Ho Yeong

(ii) TITLE OF INVENTION: TUMOR NECROSIS FACTOR RECEPTOR FAMILY PROTEIN AND RELATED NUCLEIC ACID COMPOUNDS

(iii) NUMBER OF SEQUENCES: 4

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Eli Lilly and Company
(B) STREET: Lilly Corporate Center
(C) CITY: Indianapolis
(D) STATE: Indiana
(E) COUNTRY: USA
(F) ZIP: 46202

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Cantrell, Paul R.
(B) REGISTRATION NUMBER: 36,470
(C) REFERENCE/DOCKET NUMBER: P-11586A

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (317) 276-3885
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 903 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..900

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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ATG AGG GCG CTG GAG GGG CCA GGC CTG TCG CTG CTG TGC CTG GTG TTG       48
Met Arg Ala Leu Glu Gly Pro Gly Leu Ser Leu Leu Cys Leu Val Leu
  1               5                  10                  15

GCG CTG CCT GCC CTG CTG CCG GTG CCG GCT GTA CGC GGA GTG GCA GAA       96
Ala Leu Pro Ala Leu Leu Pro Val Pro Ala Val Arg Gly Val Ala Glu
             20                  25                  30

ACA CCC ACC TAC CCC TGG CGG GAC GCA GAG ACA GGG GAG CGG CTG GTG      144
Thr Pro Thr Tyr Pro Trp Arg Asp Ala Glu Thr Gly Glu Arg Leu Val
         35                  40                  45

TGC GCC CAG TGC CCC CCA GGC ACC TTT GTG CAG CGG CCG TGC CGC CGA      192
Cys Ala Gln Cys Pro Pro Gly Thr Phe Val Gln Arg Pro Cys Arg Arg
     50                  55                  60

GAC AGC CCC ACG ACG TGT GGC CCG TGT CCA CCG CGC CAC TAC ACG CAG      240
Asp Ser Pro Thr Thr Cys Gly Pro Cys Pro Pro Arg His Tyr Thr Gln
 65                  70                  75                  80

TTC TGG AAC TAC CTG GAG CGC TGC CGC TAC TGC AAC GTC CTC TGC GGG      288
Phe Trp Asn Tyr Leu Glu Arg Cys Arg Tyr Cys Asn Val Leu Cys Gly
                 85                  90                  95

GAG CGT GAG GAG GAG GCA CGG GCT TGC CAC GCC ACC CAC AAC CGT GCC      336
Glu Arg Glu Glu Glu Ala Arg Ala Cys His Ala Thr His Asn Arg Ala
            100                 105                 110

TGC CGC TGC CGC ACC GGC TTC TTC GCG CAC GCT GGT TTC TGC TTG GAG      384
Cys Arg Cys Arg Thr Gly Phe Phe Ala His Ala Gly Phe Cys Leu Glu
        115                 120                 125

CAC GCA TCG TGT CCA CCT GGT GCC GGC GTG ATT GCC CCG GGC ACC CCC      432
His Ala Ser Cys Pro Pro Gly Ala Gly Val Ile Ala Pro Gly Thr Pro
    130                 135                 140

AGC CAG AAC ACG CAG TGC CAG CCG TGC CCC CCA GGC ACC TTC TCA GCC      480
Ser Gln Asn Thr Gln Cys Gln Pro Cys Pro Pro Gly Thr Phe Ser Ala
145                 150                 155                 160
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AGC AGC TCC AGC TCA GAG CAG TGC CAG CCC CAC CGC AAC TGC ACG GCC     528
Ser Ser Ser Ser Ser Glu Gln Cys Gln Pro His Arg Asn Cys Thr Ala
                165                 170                 175

CTG GGC CTG GCC CTC AAT GTG CCA GGC TCT TCC TCC CAT GAC ACC CTG     576
Leu Gly Leu Ala Leu Asn Val Pro Gly Ser Ser Ser His Asp Thr Leu
            180                 185                 190

TGC ACC AGC TGC ACT GGC TTC CCC CTC AGC ACC AGG GTA CCA GGA GCT     624
Cys Thr Ser Cys Thr Gly Phe Pro Leu Ser Thr Arg Val Pro Gly Ala
        195                 200                 205

GAG GAG TGT GAG CGT GCC GTC ATC GAC TTT GTG GCT TTC CAG GAC ATC     672
Glu Glu Cys Glu Arg Ala Val Ile Asp Phe Val Ala Phe Gln Asp Ile
    210                 215                 220

TCC ATC AAG AGG CTG CAG CGG CTG CTG CAG GCC CTC GAG GCC CCG GAG     720
Ser Ile Lys Arg Leu Gln Arg Leu Leu Gln Ala Leu Glu Ala Pro Glu
225                 230                 235                 240

GGC TGG GGT CCG ACA CCA AGG GCG GGC CGC GCG GCC TTG CAG CTG AAG     768
Gly Trp Gly Pro Thr Pro Arg Ala Gly Arg Ala Ala Leu Gln Leu Lys
                245                 250                 255

CTG CGT CGG CGG CTC ACG GAG CTC CTG GGG GCG CAG GAC GGG GCG CTG     816
Leu Arg Arg Arg Leu Thr Glu Leu Leu Gly Ala Gln Asp Gly Ala Leu
            260                 265                 270

CTG GTG CGG CTG CTG CAG GCG CTG CGC GTG GCC AGG ATG CCC GGG CTG     864
Leu Val Arg Leu Leu Gln Ala Leu Arg Val Ala Arg Met Pro Gly Leu
        275                 280                 285

GAG CGG AGC GTC CGT GAG CGC TTC CTC CCT GTG CAC                     903
Glu Arg Ser Val Arg Glu Arg Phe Leu Pro Val His
    290                 295                 300
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 300 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Met Arg Ala Leu Glu Gly Pro Gly Leu Ser Leu Leu Cys Leu Val Leu
 1               5                  10                  15

Ala Leu Pro Ala Leu Leu Pro Val Pro Ala Val Arg Gly Val Ala Glu
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20 25 30
Thr Pro Thr Tyr Pro Trp Arg Asp Ala Glu Thr Gly Glu Arg Leu Val
35 40 45

Cys Ala Gln Cys Pro Pro Gly Thr Phe Val Gln Arg Pro Cys Arg Arg
50 55 60

Asp Ser Pro Thr Thr Cys Gly Pro Cys Pro Pro Arg His Tyr Thr Gln
65 70 75 80

Phe Trp Asn Tyr Leu Glu Arg Cys Arg Tyr Cys Asn Val Leu Cys Gly
85 90 95

Glu Arg Glu Glu Glu Ala Arg Ala Cys His Ala Thr His Asn Arg Ala
100 105 110

Cys Arg Cys Arg Thr Gly Phe Phe Ala His Ala Gly Phe Cys Leu Glu
115 120 125

His Ala Ser Cys Pro Pro Gly Ala Gly Val Ile Ala Pro Gly Thr Pro
130 135 140

Ser Gln Asn Thr Gln Cys Gln Pro Cys Pro Pro Gly Thr Phe Ser Ala
145 150 155 160

Ser Ser Ser Ser Ser Glu Gln Cys Gln Pro His Arg Asn Cys Thr Ala
165 170 175

Leu Gly Leu Ala Leu Asn Val Pro Gly Ser Ser Ser His Asp Thr Leu
180 185 190

Cys Thr Ser Cys Thr Gly Phe Pro Leu Ser Thr Arg Val Pro Gly Ala
195 200 205

Glu Glu Cys Glu Arg Ala Val Ile Asp Phe Val Ala Phe Gln Asp Ile
210 215 220

Ser Ile Lys Arg Leu Gln Arg Leu Leu Gln Ala Leu Glu Ala Pro Glu
225 230 235 240

Gly Trp Gly Pro Thr Pro Arg Ala Gly Arg Ala Ala Leu Gln Leu Lys
245 250 255

Leu Arg Arg Arg Leu Thr Glu Leu Leu Gly Ala Gln Asp Gly Ala Leu
260 265 270

Leu Val Arg Leu Leu Gln Ala Leu Arg Val Ala Arg Met Pro Gly Leu
275 280 285

Glu Arg Ser Val Arg Glu Arg Phe Leu Pro Val His
290 295 300

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 59 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TAGGGCTGAT CAAGGATGGG CTTCTGGACT TGGGCGGCCC CTCCGCAGGC GGACCGGGG 59

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 66 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

AGGGGGGCGG CCGCTGATCA TCACTTGTCG TCGTCGTCCT TGTAGTCGTG CACAGGGAGG 60

AAGCGC 66

WE CLAIM:

1. A substantially pure protein comprising an amino acid sequence which is amino acids 34-195 of SEQ ID NO:2.

2. The substantially pure protein of claim 1 comprising an amino acid sequence which is amino acids 30-300 of SEQ ID NO:2.

3. The substantially pure protein of claim 1 comprising an amino acid sequence which is SEQ ID NO:2.

4. An isolated nucleic acid compound encoding the protein of Claim 1, or a sequence complementary to said compound.

5. An isolated nucleic acid compound encoding the protein of claim 2, or a sequence complementary to said compound.

6. An isolated nucleic acid compound encoding the protein of Claim 3, or a sequence complementary said molecule.

7. An isolated nucleic acid compound encoding a protein having osteoclast differentiation-inhibiting activity, wherein said compound hybridizes to a nucleic acid compound as set forth in SEQ ID NO:1 under high stringency conditions.

8. The isolated nucleic acid compound of claim 7 that is at least 75% identical to a nucleic acid compound as set forth in SEQ ID NO:1.

9. A vector comprising the isolated nucleic acid compound of Claim 4.

10. The vector of Claim 9, wherein said isolated nucleic acid compound is SEQ ID NO:1 operably-linked to a promoter sequence.

11. A host cell transformed with a vector of Claim 9.

12. A host cell transformed with a vector of Claim 10.

13. A method for constructing a recombinant host cell having the potential to express a protein comprising an amino acid sequence consisting of amino acids 34-195 of SEQ ID NO:2, said method comprising introducing into said host cell by any suitable means a vector of claim 10.

14. A method for expressing a protein identified herein as SEQ ID NO:2 in a recombinant host cell of Claim 13, said method comprising culturing said recombinant host cell under conditions suitable for gene expression.

15. A method for identifying compounds that bind a protein identified herein as SEQ ID NO:2, comprising the steps of:

a) admixing a substantially purified preparation of a protein comprising SEQ ID NO:2 with a test compound; and

b) monitoring by any suitable means a binding interaction between said protein and said compound.

16. A method, as in Claim 15 wherein said protein is identified herein as SEQ ID NO:2.

17. An antibody that selectively binds to a protein identified herein as SEQ ID NO:2, or fragment thereof.

18. The isolated nucleic acid of Claim 4 as set forth in SEQ ID NO:1.

19. The isolated nucleic acid of Claim 4 corresponding to nucleotides 88-900 of SEQ ID NO:1.

20. The isolated nucleic acid of Claim 4 corresponding to nucleotides 102-585 of SEQ ID NO:1.

21. The isolated TNFRsol/OPG3 polypeptide comprising an amino acid sequence at least 95% identical to a sequence selected from the group consisting of:

(a) the amino acid sequence having the sequence shown as SEQ ID NO:2;

(b) the amino acid encoded by the nucleotide sequence shown as SEQ ID NO:1;

(c) the amino acid sequence having the amino acids 34-195 of the sequence shown as SEQ ID NO:2;

(d) the amino acid sequence having the amino acids 30-300 of the sequence shown as SEQ ID NO:2.

(e) the amino acid sequence encoded by the nucleotide sequence shown as nucleotides 88-900 of the sequence shown as SEQ ID NO:1; and

(f) the amino acid sequence encoded by the nucleotide sequence shown as nucleotides 102-585 of the sequence shown as SEQ ID NO:1.

22. A method for treating a patient in need of TNFRsol/OPG3 polypeptide activity comprising administering to said patient an effective amount of the polypeptide of claim 21.

23. The method of claim 22 wherein the patient is being treated for hepatitis, sepsis, renal failure, hepatocellular injury, cardiac ischemia, neuronal ischemia, transplantation rejection, allergies, HIV, colon carcinoma, melanoma,

hepatocellular carcinoma, lung cancer, astrocytoma, esophageal carcinoma, IDDM, multiple sclerosis (MS), rheumatoid arthritis and lupus.

24. A method for modulating selected TNFR interactions with their respective TNFR ligands comprising administering and effective amount of the polypeptide of claim 21.

25. The method of claim 24 wherein the TNFR family ligands are selected from the group consisting of FasL and LIGHT.

26. A method for modulating selected TNFR interactions with their respective TNFR family ligands *in vitro* comprising administering an effective amount of the polypeptide of claim 21.

27. A method for modulating selected TNFR interactions with their respective TNFR family ligands *ex vivo* comprising administering an effective amount of the polypeptide of claim 21.

28. The method of claim 27 further comprising administering to cells undergoing gene therapy.

29. The method of claim 26 wherein the TNFR family ligands are selected from the group consisting of FasL and LIGHT.

30. The method of claim 27 wherein the TNFR family ligands are selected from the group consisting of FasL and LIGHT

31. A method for modulating modulating selected TNFR interactions with their respective TNFR family ligands interaction in a mammal comprising administering an effective amount of the polypeptide of claim 21.

32. The method of claim 31 wherein the mammal is human.

33. A method for modulating FasL mediated apoptosis *in vitro* comprising providing a sample containing cells which undergo FasL mediated apoptosis and contacting said sample with an effective amount of the polypeptide of claim 21.

34. The method of claim 24 wherein the modulation is down regulation of FasL mediated apoptosis.

35. The method of claim 24 wherein the modulation is upregulation LIGHT mediated cell proliferation.

36. The method of claim 24 wherein the selected TNFR interactions with their respective TNFR ligands occurs in cells selected from the group consisting of T cells, myocytes, renal tubule epithelial cells (RTC), neutrophils, neurons, thyrocytes, stroma cells, acinar cells, Sertoli cells, macrophages, hepatocytes, leukemia cells, cells of the kidney cortex and tumor cells.

37. The method of claim 32 wherein said T cells are activated.

38. The method of claim 32 wheren the macrophages are infected with Human immunodeficiency virus (HIV).

39. The method of claim 36 wherein said heptocytes have been treated with ethanol.

40. The method of claim 36 wherein said leukemia cells have been exposed to chemotherapy drugs.

41. A method for treating conditions in a mammal which conditions are caused or exacerbated by FasL mediated apoptosis comprising identifying a mammal suffering from a condition caused or exacerbated by FasL mediated apoptosis

and administering an effective amount of the polypeptide of claim 21.

42. The method of claim 41 wherein said conditions are selected from the group consisting hepatitis, sepsis, renal failure, hepatocellular injury, cardiac ischemia, neuronal ischemia, transplantation rejection, allergies, HIV, colon carcinoma, melanoma, hepatocellular carcinoma, lung cancer, astrocytoma, esophageal carcinoma, IDDM, multiple sclerosis (MS), rheumatoid arthritis and lupus.

43. A method of preventing or reducing hepatocellular damage in a mammal comprising administering an effective amount of the polypeptide of claim 21.

44. A method of preventing or reducing tubular atrophy in renal failure in a mammal comprising administering an effective amount of the polypeptide of claim 21.

45. A method of treating hepatitis in a mammal comprising administering an effective amount of the polypeptide of claim 21.

46. The method of claims 22 or 41 further comprising administering antiinflammatory drugs or steroids

ABSTRACT

The invention provides isolated nucleic acid compounds, proteins, and fragments thereof, said proteins being related to the family of tumor necrosis factor receptors. Also provided are vectors and transformed heterologous host cells for expressing the protein and a method for identifying compounds that bind and/or modulate the activity of said proteins. Also provided are method for treating disease by modulating interactions and/or binding between members of the TNFR superfamily and their respective ligands.